IMMUNOHISTOLOGIC STUDIES OF TUMORS CONTAINING MYOSIN

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The identification of rhabdomyosarcomas is sometimes difficult or impossible when certain differentiating characteristics are not present.[1,2] We previously investigated 6 specimens of rhabdomyosarcoma and found that the embryonal types contained myosin and the pleomorphic types did not.[3] Since Wilms's tumor often contains skeletal muscle cells, immunohistochemical methods were used to identify muscle in both Wilms's tumor and rhabdomyosarcomas. The use of immuno-specific staining as an aid to histologic diagnosis is rapid, simple to perform and can be of particular importance in confirming the identification of embryonal rhabdomyosarcoma and Wilms's tumor.

Additional studies with embryonal rhabdomyosarcoma confirming the earlier results are presented in addition to the application of this technique to 4 Wilms's tumors.

MATERIAL AND METHODS

Preparation of Antisera

A transplantable mouse rhabdomyosarcoma was obtained from Dr. V. Suntzeff, Washington University Medical School, St. Louis, Mo. The tumor was grown for 3 weeks subcutaneously on the backs of C_3H mice and harvested under sterile conditions. The tissues were examined grossly and dissected away from necrotic elements and visible connective tissues. Tumor tissues were frozen immediately in dry ice and stored at $-20°$ C. Two gm frozen, pooled rhabdomyosarcoma from these animals was homogenized with 4 ml of saline solution in a Servall Omnihomogenizer in an ice bath at full speed for 1 minute. The homogenate was strained through sterile #40 steel mesh and emulsified manually in a Pyrex glass homogenizer with 4 ml complete Freund's adjuvant until a thick, creamy consistency was obtained. The adjuvant was prepared using 5 parts Bayol 55 to 1 part Arlacel A and 1 mg per ml of *Mycobacterium butyricum* (Difco Laboratories, Detroit, Mich.).

The adjuvant mixture was injected into 2 young adult New Zealand rabbits as follows: 0.2 ml intramuscularly into each thigh and 0.2 ml subcutaneously into the back (0.6 ml total). Injections were repeated after 4 weeks, and the animals were allowed to rest for 4 weeks. They were then bled and the serum tested.

The animals were reinjected with 0.5 ml into each site (1.5 ml total) every 2 weeks, with bleedings taken on alternate weeks. The rabbit anti-mouse rhabdomyosarcoma sera were pooled from the various bleedings and employed for study.

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This work was supported in part by the American Lebanese Syrian Associated Charities (ALSAC).

Accepted for publication, July 7, 1965.

Goat anti-rabbit globulin serum was prepared in 4 goats by injecting each animal with 10 mg per ml of rabbit globulin in Freund's adjuvant, 1 ml per site into 4 sites intramuscularly. Four weeks later the same goats were each given intramuscular injections of rabbit globulin-Freund's adjuvant preparation, using 2 ml of the preparation per injection at 4 different sites. Four weeks following the second intramuscular injections, the same goats were each given 20 ml of 2.5 mg per ml rabbit globulin solution intravenously. Thus, a total of 3 series of injections per animal were given in 8 weeks. The animals were bled 1 week after the last injection. Precautions were taken against anaphylactic shock.

The rabbit anti-human myosin serum preparation has been described previously.[3] In brief, human skeletal muscle from necropsies was ground in the cold and extracted with a mixture of KCl and potassium phosphate. After dilution with cold water, the mixture was strained through gauze and further aqueous dilutions made. After standing in the cold, the supernate was removed and the precipitate collected by centrifugation. The protein was dissolved in KCl plus a solution of K_2PO_4 and KH_2PO_4. Aqueous dilution was made (pH 6.5 to 6.8) and the solution was clarified by centrifugation and filtration. More water was added and the myosin precipitate removed by centrifugation. The myosin was precipitated by adding cold water and the myosin was dissolved in a solution of KCl and dialyzed. The myosin thusly obtained was used to inject into rabbits, utilizing the same procedure as outlined above for the preparation of rabbit anti-mouse rhabdomyosarcoma serum.

Both the rabbit anti-human myosin sera and rabbit anti-mouse rhabdomyosarcoma sera were treated with human liver sediments to remove antibodies that might react with normal connective tissue. The antisera, following this absorption technique, did not react with normal human liver, spleen or kidney but did react with human skeletal muscle (Table I). Figures 1 and 2 show the reactions obtained when each of the antisera was employed for staining of normal human fetal muscle cells. These immunospecific reagents were used to study the embryonal rhabdomyosarcomas from 4 individuals. In addition, neoplastic tissues from 4 other patients with Wilms's tumor were studied by the same procedure.

Source of Tissue

Normal human skeletal muscle, liver and spleen for control staining were obtained from necropsies performed upon young adults within 6 hours following sudden death in traffic accidents.

Small samples of the rhabdomyosarcomas and Wilms's tumors were taken immediately after removal in the operating room. These samples were bisected, one portion being placed in formalin for histologic processing and the other portion frozen without delay at $-20°$ C and stored for fluorescent antibody staining. At least 3 samples taken from various areas of each tumor were prepared in this manner. Tissues from these 2 types of neoplasm obtained at necropsy were treated in precisely the same fashion. The anatomic location of the sample sites are given in the synopsis of each case in the Results.

Technique of Staining

Multiple sections, 5 μ thick, of the frozen tumor tissues ($-20°$ C) were prepared in a cryostat, placed on pre-cooled glass slides, dried for 15 minutes at room temperature. They were then moistened with saline, treated with 2 drops of rabbit anti-human myosin serum for 1 hour in a moist atmosphere chamber, washed by gentle shaking in saline for 10 minutes, treated with 2 drops of fluorescein-tagged goat anti-rabbit globulin serum for 1 hour in a moist chamber and finally mounted in glycerin under a glass cover slip and examined with an ultraviolet light microscope for fluorescence. These techniques have been described in previous publications.[4] The same method was used in testing the human tumors with fluorescein-tagged anti-mouse rhabdomyosarcoma serum. The histologic preparations of each sample

of tumor as well as normal controls were compared with the immunohistologic counterpart of the originally bisected sample, and photographs of each were taken.

RESULTS

Two rabbit antisera were employed for the rapid detection of muscle elements in human rhabdomyosarcoma and Wilms's tumor. Specific staining was manifestly confined to muscle cells which were seen arranged in typical fascicles. Any antibody activity against the connective tissues was effectively removed by absorbing the antiserum with human liver and spleen sediments.

The following individual descriptions of cases with embryonal rhabdomyosarcomas and 4 with Wilms's tumors include the histologic features, pertinent clinical data and presence or absence of positive immunofluorescence.

Embryonal Rhabdomyosarcoma

A 21-month-old girl was found to have polypoid vaginal masses typical of sarcoma botryoides with the histologic features of embryonal rhabdomyosarcoma (Figs. 3 and 4). Many "strap" shaped muscle cells were present as well as scattered "tadpole" and giant cells, but striated cells were few. Frozen sections were made from this specimen and stained by the fluorescent antibody method. A large concentration of myosin in the tumor cells was indicated by the strongly positive reactions to both anti-mouse rhabdomyosarcoma and anti-human myosin sera (Figs. 5 and 6). Sections from various portions of the neoplasm were all similarly positive.

A 15-year-old boy developed swelling of the right facial soft tissues which was originally diagnosed as a neuroblastoma, but later classified as an embryonal rhabdomyosarcoma. The histologic features of the primary tumor are shown in Figure 7. He expired 6 months later of extensive central nervous system metastases to the subarachnoid space. The tumor had entered the cranial vault via the foramina ovale and spinosum. Specimens of the tumor tissue exhibited strongly positive reactions to both anti-human myosin and anti-mouse rhabdomyosarcoma sera by the indirect fluorescent antibody method (Figs. 8 and 9).

S. M. was a 6-year-old girl when the onset of symptoms began with nasopharyngeal inflammation and edema. A diagnosis of embryonal rhabdomyosarcoma of the soft tissues of the nasopharynx and face was made 6 months later (Fig. 10). Six months following diagnosis she expired of massive tumor compression of the pharyngo-laryngeal and cervical tissues. Frozen sections of the tumor specimen exhibited strongly positive fluorescence when tested with anti-human myosin and with anti-mouse rhabdomyosarcoma sera (Table I).

A 3-year-old boy had right facial swelling. Original biopsy tissue was first considered to be transitional cell carcinoma but later diagnosed as embryonal rhabdomyosarcoma (Fig. 11). He expired 2 years later with brain and lung metastases. Dr. A. P. Stout, Pathologist, College of Physicians and Surgeons, Columbia University, New York, and Dr. R. C. Horn, Pathologist, Henry Ford Hospital, Detroit, concurred in the diagnosis of embryonal rhabdomyosarcoma. However, repeated attempts failed to demonstrate any positive anti-human myosin or anti-mouse rhabdomyosarcoma staining in the primary or metastatic tumors (Table I).

Wilms's Tumor (Nephroblastoma)

A 4-year-old boy was diagnosed as having nephroblastoma. The nephrectomy specimen revealed a typical Wilms's tumor including striated muscle cells (Fig. 13).

Table I

Histochemical Staining Reagents	Embryonal Rhabdomyosarcoma: T. R. (Vagina)	Embryonal Rhabdomyosarcoma: R. B. (Face)	Embryonal Rhabdomyosarcoma: S. M. (Biopsy: pharynx)	Embryonal Rhabdomyosarcoma: S.M. (Necropsy: pharynx, larynx and neck)	Embryonal Rhabdomyosarcoma: J. G. (Face and pulmonary metastases)	Wilms's Tumor: S.H. (Kidney)	Wilms's Tumor: B.P. (Kidney)	Wilms's Tumor: B.M. (Kidney)	Wilms's Tumor: B.M. (Mesenteric metastasis)	Wilms's Tumor: N.J. (Recurrent, renal fossa)	Wilms's Tumor: N.J. (Liver metastasis)	Normal Tissue: Normal skeletal muscle	Normal Tissue: Normal spleen	Normal Tissue: Normal liver
Anti-human myosin	+	+	+	+	−	−	−	+	+	+	+	+	−	−
Anti-mouse rhabdomyosarcoma	+	+	+	+	−	−	−	+	+	+	+	+	−	−
Hematoxylin and eosin Gomori's trichrome Phosphotungistic acid-hematoxylin (1) Histologic identification of muscle cells based on structure only ("tadpole" cells, "racket-shaped" cells, eosinophilic cytoplasmic nuclei).	+	+	+	+	+	+	−	+	+	+	+	+		
(2) Histologic identification of muscle cells by presence of striations in sections.	+	−	−	−	−	−	−	+	−	+	−	+		

Laparotomy was performed 4 times in the following 4 years to relieve intestinal or biliary obstruction secondary to recurrent tumor. Microscopic examination of tissue removed during each surgical procedure revealed an increasing percentage of a cell type morphologically resembling that present in some embryonal rhabdomyosarcoma (Fig. 12; compare with Fig. 7). The entire tumor consisted of this cell type at necropsy 4 years after diagnosis. Cross striations were not found in it at necropsy. However, both anti-human myosin and anti-mouse rhabdomyosarcoma sera exhibited brilliant fluorescence when tested against the tumor cells by the method described above (Figs. 14 and 15).

The diagnosis of nephroblastoma was made in an 18-year-old girl. The nephrectomy specimen contained striated muscle cells within the neoplasm (Fig. 16). The second surgical procedure revealed tumor recurrence at the primary site and liver metastasis. Tissues from both sites were composed predominantly of a dense, whorled pattern of cells resembling fibrosarcoma (Fig. 17). Gomori's trichrome staining of the cytoplasm suggested the presence of muscle cells. Sections of the hepatic metastases fluoresced strongly when tested with both anti-mouse rhabdomyosarcoma and anti-human myosin sera (Figs. 18 and 19).

A 5½-year-old girl had a nephrectomy performed for Wilms's tumor. The majority of cells in the neoplasm were densely packed with round to oval, darkly stained nuclei. Very little cytoplasm was present in each cell. A few foci of pseudotubules were present (Fig. 20). There was no morphologic or histochemical evidence of muscle cells in sections of the tissue obtained at the time of nephrectomy. This was also the case in sections studied from tissue obtained in a subsequent partial nephrectomy for tumor in the opposite kidney. Sections of tissue from the original nephrectomy were not stained by anti-mouse rhabdomyosarcoma or anti-human myosin sera (Table I).

A diagnostic biopsy of a flank mass revealed Wilms's tumor in a 5-year-, 7-months-old girl. Numerous pseudo-tubules were present (Fig. 21). At this time many densely packed cells with little cytoplasm and round to oval darkly staining nuclei were noted. Following radiotherapy and chemotherapy for 3 months, nephrectomy was performed. The majority of cells in the nephrectomy specimen were much larger than those in the biopsy tissue. While no cytoplasmic striations were observed, the cells resembled those sometimes found in embryonal rhabdomyosarcoma and in some cases of Wilms's tumor (Fig. 22; compare with Figs. 7 and 12). Trichrome stains also suggested that these were muscle cells rather than fibroblasts. Despite these histologic and histochemical findings, reaction of tumor tissue from the nephrectomy tissue was negative with anti-mouse rhabdomyosarcoma and with anti-human myosin sera (Table I).

Histologically, all of the embryo rhabdomyosarcomas showed some evidence of embryonal muscle origin, with only one of the rhabdomyosarcoma specimens revealing the presence of muscle striations by conventional histochemical staining. Three of the 4 embryonal rhabdomyosarcomas reacted with the anti-mouse rhabdomyosarcoma serum and with the anti-human myosin serum in a strongly positive manner.

Immunohistochemically, strong staining was visualized in the cytoplasm of many sarcomatous appearing cells. Some spindle shaped cells likewise fluoresced intensely. Large cell nests as well as scattered individual cells were rapidly identified by this procedure. Intense fluorescence was noted at the peripheral margins of many of the positively staining cells. The connective tissues surrounding many of these cell nests were

unstained. In the fourth case with embryonal rhabdomyosarcoma the course of the disease was typical and the tissue had a classic histologic appearance. Nevertheless the tissue did not stain with anti-mouse rhabdomyosarcoma or anti-human myosin sera by either the direct or indirect methods. Numerous sections were made to insure adequate sampling of the tumor but in no section could positive staining of muscle elements be demonstrated with the anti-myosin sera.

Primary and metastatic tumor tissues from 2 of the 4 Wilms's tumors reacted positively to anti-mouse rhabdomyosarcoma serum and to anti-human myosin serum.

There was corroborative evidence of the presence of muscle cells by histologic appearance alone in the early stages of development in both of the Wilms's tumors by conventional histologic studies. Cytoplasmic cross striations were demonstrated in tissue from both in either the primary or locally recurring tumor, but not in metastases. The other 2 Wilms's tumors gave completely negative reactions to anti-mouse rhabdomyosarcoma serum and to anti-human myosin serum. In these 2 Wilms's tumors, 1 possessed cells resembling immature muscle cells in conventional histologic preparations, but no cytoplasmic cross striations were found in either (Table I).

Discussion

Preliminary studies of human rhabdomyosarcoma using immunospecific reagents showed that despite the anaplastic nature of the neoplasm, myosin could be detected in some but not in all embryonal rhabdomyosarcomas. In this study 4 embryonal rhabdomyosarcomas and 4 Wilms's tumors were studied using two different antibodies. We were able to demonstrate that the anti-human myosin serum was capable of specifically staining muscle cells. A second serum, rabbit anti-mouse rhabdomyosarcoma serum, also stained human muscle elements. This cross reaction of the antiserum was used to detect human embryonal muscle tumors. Of the 4 tumors classified as embryonal rhabdomyosarcoma, 3 were positive by the fluorescent antibody methods while only 1 of these 3 showed the presence of muscle striations by special stains. In the 4 cases of Wilms's tumor investigated, 2 were positive by the fluorescent antibody staining technique. These 2 were also positive for muscle striations. One Wilms's tumor in which primitive muscle cells and muscle striations were not recognized histologically, was likewise negative by the fluorescent antibody method.

One Wilms's tumor and 1 embryonal rhabdomyosarcoma contained cells with the appearance of primitive muscle but no striations were seen. The fluorescent antibody procedure in both instances was negative. The reasons for this difference might have been (1) in sampling, (2) that

the cells were not primitive muscle cells, and (3) that the cells were lacking certain muscle antigens. Although selection of representative and viable areas of a given tumor is often inadequate, in these instances it appeared unlikely that inadequate sampling occurred since adjacent sections were studied by histologic staining and fluorescent antibody methods. The possibility that the cells may not have been of primitive muscle origin is difficult to establish, and at the present time cannot be completely eliminated from consideration. The fact that the cells did have the same characteristics in terms of structure and staining affinity for certain dyes indicates that they may have been primitive muscle cells. It may be that the cells had deleted certain antigens such as myosin, and these would not be expected to demonstrate positive staining for this substance.[5] The fact that tumor cells lose specific antigens has been demonstrated by Weiler.[6] A more recent study on reduction of normal muscle antigen in tumors of muscle origin has been published by Fel and Tsikarishvili.[7] We have also observed this to some degree in carcinogen induced hepatomas.[8]

In view of the difficulties associated with diagnosing certain neoplastic tissues, the use of an immunospecific reagent is helpful. An immunospecific reagent can be made against any number of functional proteins or products being synthesized by a normal cell, whether it be myosin,[9] insulin[10] or nerve growth factors.[11] Such a reagent can then specifically detect a functional capacity in a tumor or a distinct product associated only with certain types of normal cells, namely, muscle, islet cells of the pancreas or salivary gland cells. By this process it is possible to identify certain anaplastic cells and classify the neoplasm. That neoplasms may delete antigens has been documented and should be recognized. The fact that myosin is no longer being synthesized by a rhabdomyosarcoma, however, does not necessarily mean that other specific muscle proteins are not present. Therefore, the further development of new immunospecific reagents to other functional normal muscle antigens is warranted.

In addition to the specificity, the application of the procedure is simple and rapid once the characteristics of the reagents are established. Using the sensitive, indirect staining method on fresh frozen sections, detection for muscle elements can be completed within 3 hours after the specimen is received. Furthermore, the detection of these procedures does not require that the anaplastic cells conform to certain morphologic types, nor is presence of muscle striations required.

Summary

Antibodies to myosin, a protein present in embryonal rhabdomyosarcoma, were developed by injecting into rabbits myosin extracted from

normal human skeletal muscle. Fluorescein-tagged goat anti-rabbit-globulin globulin was obtained and used in the indirect immunofluorescent method on frozen sections to study 4 embryonal rhabdomyosarcomas. Three of these exhibited positive immunofluorescence. Two of 4 Wilms's tumors also revealed groups or immunofluorescent positive cells. Skeletal muscle cells were found in the same 2 tumors by light microscopy. Mouse rhabdomyosarcoma exhibited positive immunofluorescence by this technique. Positive controls consisted of normal human skeletal muscle. Normal human liver and spleen were used as negative controls.

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[*Illustrations follow*]

Legends for Figures

Fig. 1. Cross section of human fetal muscle treated with rabbit anti-human myosin serum and then stained with fluorescein labeled goat anti-rabbit gamma globulin. Staining is confined to the muscle bundles only. Stromal elements separating such bundles are completely unstained. × 140.

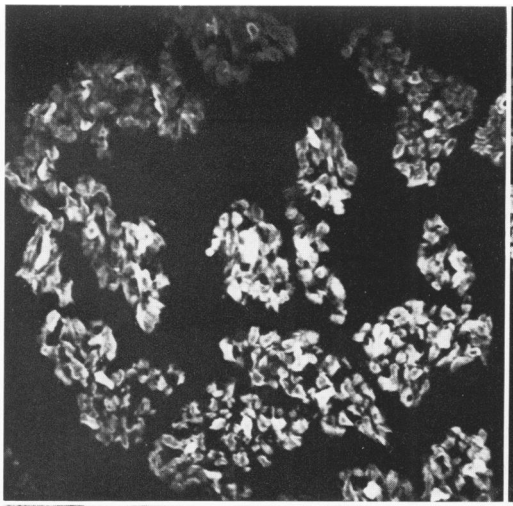
Fig. 2. Section taken from the same control muscle shown in Figure 1, but treated with rabbit anti-mouse embryonal rhabdomyosarcoma serum instead of rabbit anti-human myosin serum. Muscle cells clearly give a positive reaction and the fasicles are separated by negatively staining endo- and perimysial connective tissue. × 140.

Fig. 3. Typical polypoid structure of a sarcoma botyroides (embryonal rhabdomyosarcoma) which arose from the vaginal wall. Hematoxylin and eosin (H&E) stain. × 25.

Fig. 4. A higher magnification of the lesion shown in Figure 3 demonstrates typically elongated, neoplastic embryonal muscle cells scattered and in bundles within very sparse connective tissue. A few tumor cells exhibited cytoplasmic cross striations with special stains. H&E stain. × 190.

Fig. 5. Frozen sections of the specimen shown in Figures 3 and 4 were treated with rabbit anti-human myosin sera and subsequently stained with fluorescein labeled goat anti-rabbit globulin reagent. Myosin-containing neoplastic elements fluoresce brilliantly. Connective tissues were completely negative. × 140.

Fig. 6. Sections as in Figure 5 treated with rabbit anti-mouse rhabdomyosarcoma serum and subsequently stained with fluorescein labeled goat anti-rabbit gamma globulin reagent. Elongated embryonic muscle-like cells are stained indicating the presence of myosin. The staining is confined to structures morphologically similar to those seen in Figure 5. × 140.



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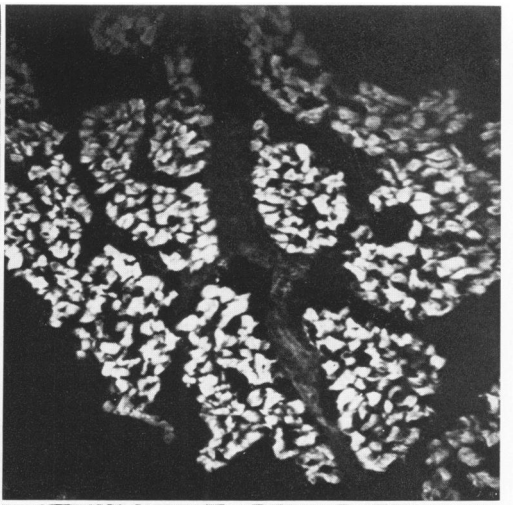

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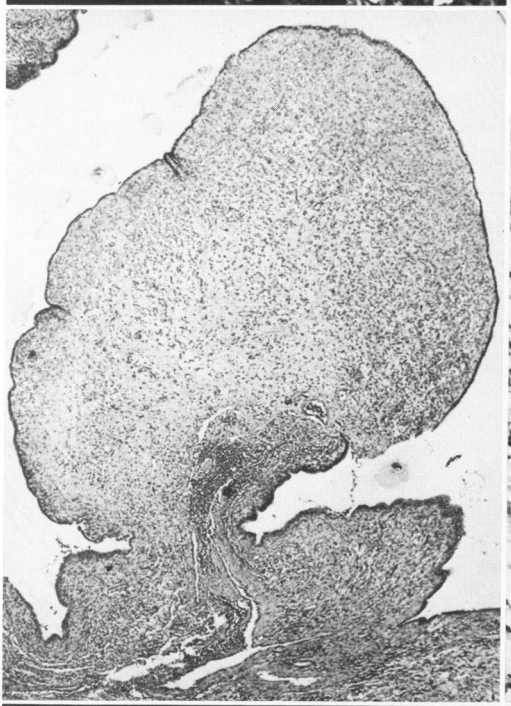

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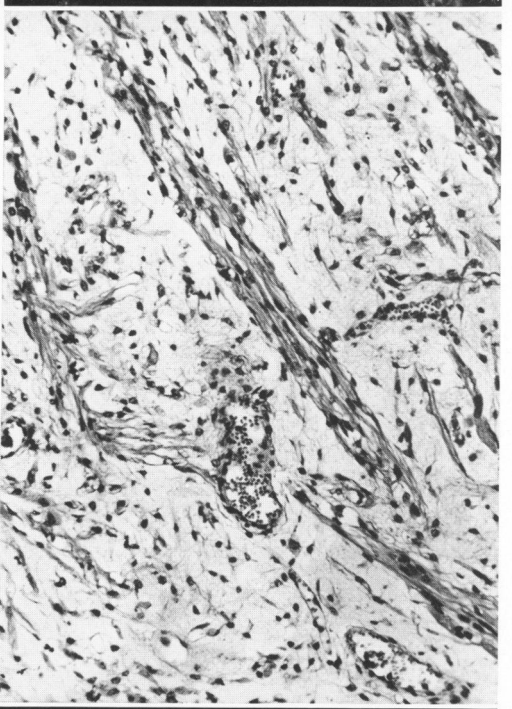

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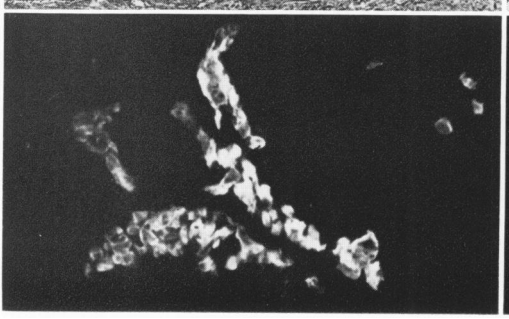

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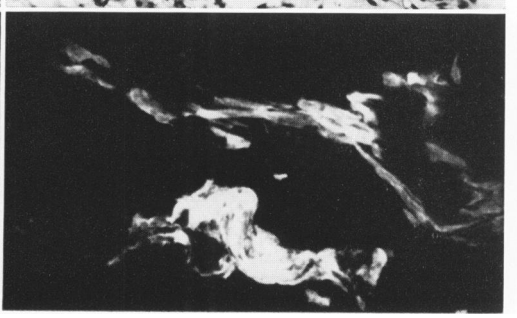

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FIG. 7. Embryonal rhabdomyosarcoma from the buccal soft tissues. There are many large round to oval embryonic muscle cells, most of which contain large nuclei. Also present are smaller, more pleomorphic tumor cells less obviously of myogenic origin. H&E stain. × 160.

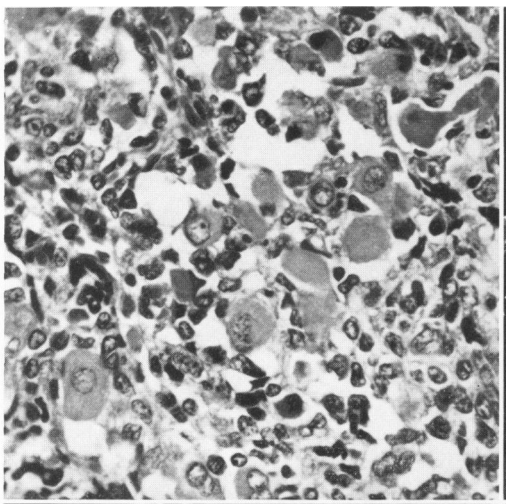
FIG. 8. Section of the same tumor shown in Figure 7 was treated with rabbit anti-human myosin serum and subsequently stained with goat anti-rabbit conjugate. Staining is confined to large sarcoma-like cells. Intense fluorescence can be seen confined to the peripheral margins of many of the positively staining cells. Some of the pleomorphic tumor cells demonstrate large nuclei. × 140.

FIG. 9. Sections from the tissue seen in Figure 7 treated with rabbit anti-mouse rhabdomyosarcoma and subsequently stained with goat anti-rabbit conjugate. The staining reaction is very similar to that seen in Figure 8. × 140.

FIG. 10. Embryonal rhabdomyosarcoma of the pharyngeal and facial soft tissues. Pleomorphism, nuclear staining variation and one huge tumor giant cell are shown. H&E stain. × 160.

FIG. 11. A representative area of a pulmonary metastasis from a rhabdomyosarcoma arising in the cheek. Pleomorphic and bizarre tumor giant cells contain large amounts of eosinophilic cytoplasm. No cytoplasmic cross-striations were found with special staining. H&E stain. × 160.

FIG. 12. All tissue from the necropsy of this patient with nephroblastoma showed this one general cell type with the cytoplasm staining pink with eosin and red by Gomori's trichrome stain. No cross striations were found with phosphotungstic acid hematoxylin stains (compare with Figures 7 and 22). H&E stain. × 320.



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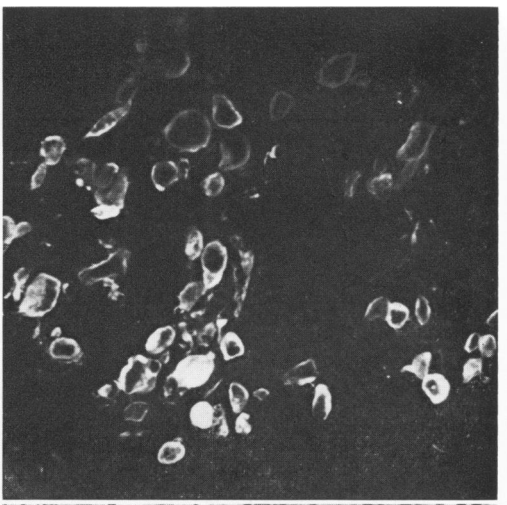

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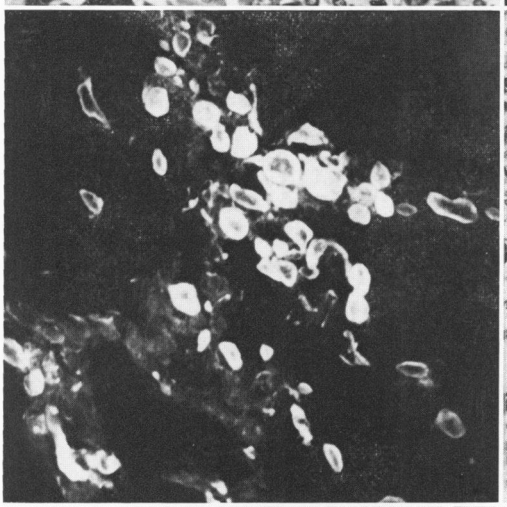

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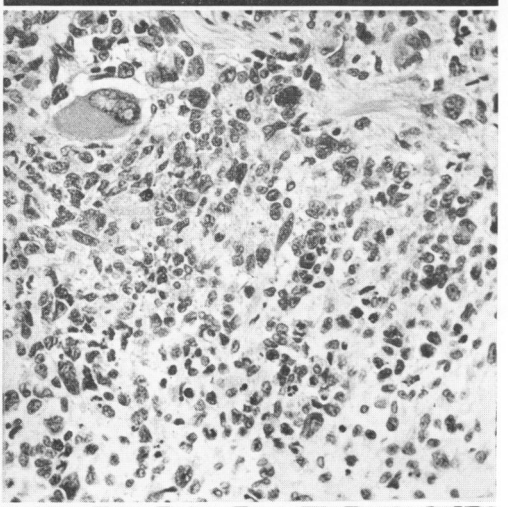

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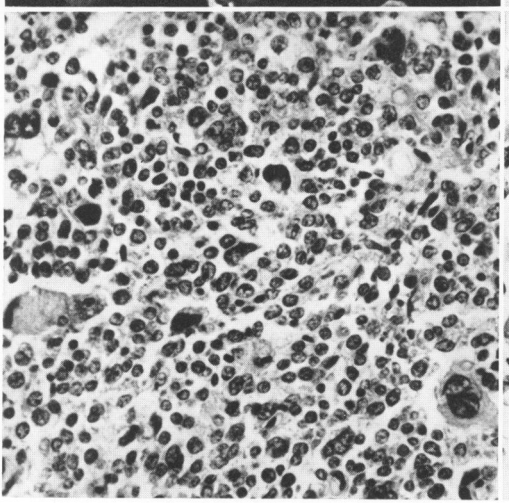

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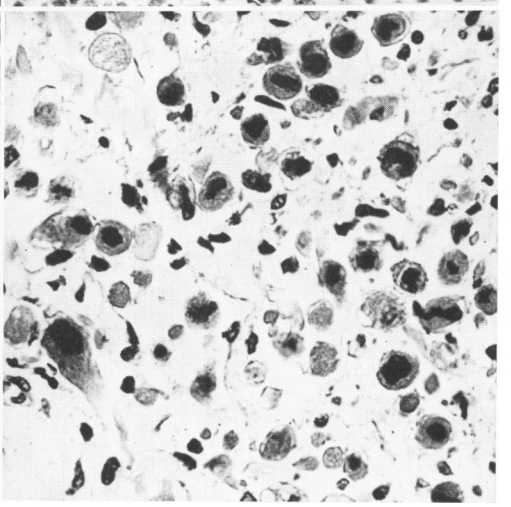

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FIG. 13. Tissue from the original nephrectomy specimen for nephroblastoma in the case exhibited in Figure 12. Cross striations appear in the cytoplasm of tumor cells. × 900.

FIG. 14. Frozen section of the specimen shown in Figure 12, treated with rabbit anti-human myosin serum and subsequently stained with goat anti-rabbit conjugate. Intense staining is confined to elongated muscle-like elements. Other presumably neoplastic cells not containing myosin are unstained. × 140.

FIG. 15. Frozen section of tissue shown in Figure 12, treated with rabbit anti-mouse rhabdomyosarcoma and subsequently stained with goat anti-rabbit conjugate. Staining is confined to elongated muscle-like cells as in Figure 14. The specificity of the reaction is indicated by the completely negative background. × 140.

FIG. 16. Tissue from a nephrectomy specimen with nephroblastoma. Cross striations are shown in the cytoplasm of elongated cells. Phosphotungstic acid hematoxylin stain. × 900.

FIG. 17. Hepatic metastasis, following radiation and chemotherapy from the case seen in Figure 16. There is evolution toward a sarcomatous pattern which resembles either fibro- or leiomyosarcoma. H&E stain. × 160.

FIG. 18. Hepatic metastases from the case shown in Figure 16 were treated with rabbit anti-human myosin serum and subsequently stained with fluorescein labeled goat anti-rabbit globulin. Three elongated myosin containing tumor cells fitting the description given in Figure 17 are demonstrated. × 140.

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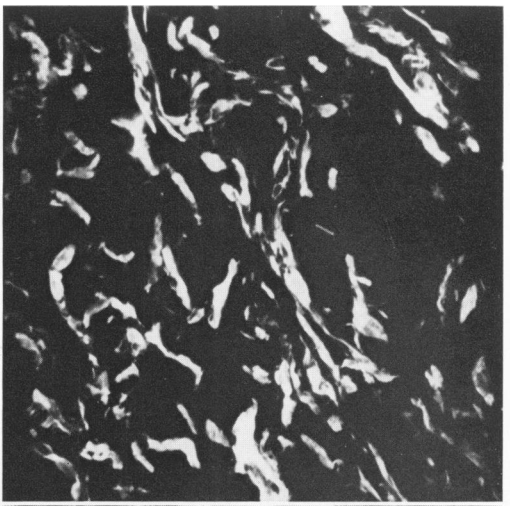

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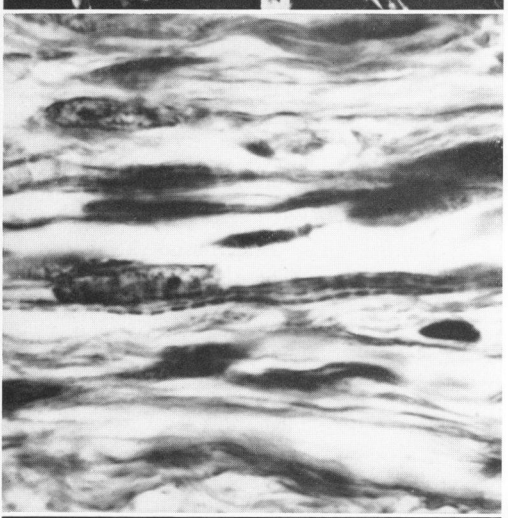

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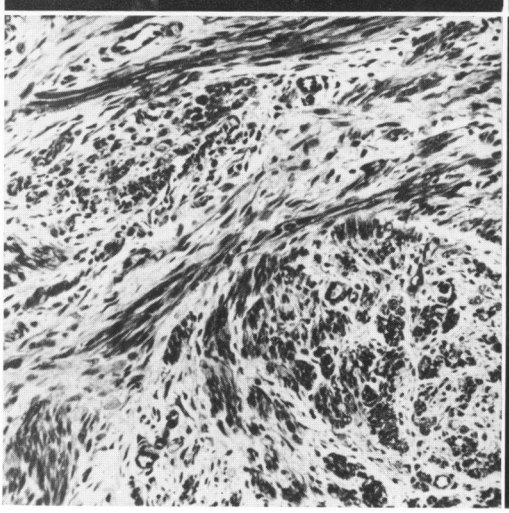

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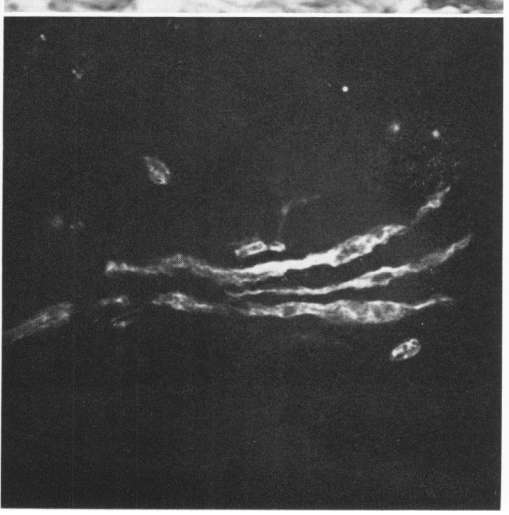
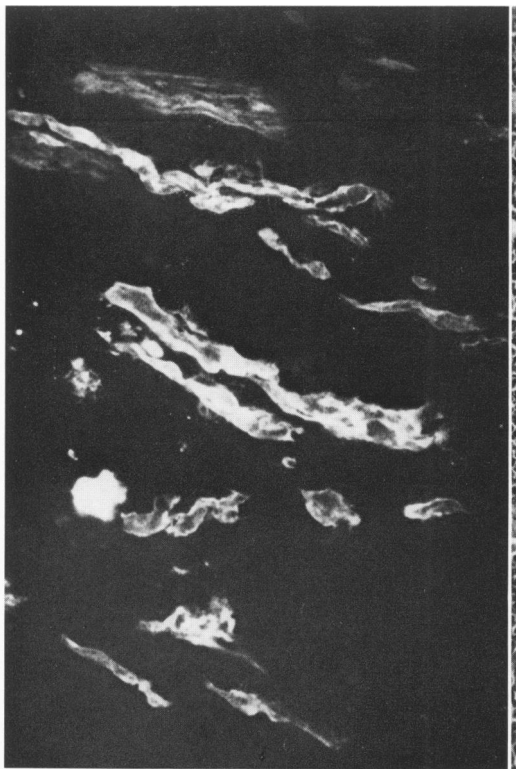


FIG. 19. Liver metastasis in the case shown in Figure 17 was treated with rabbit anti-mouse rhabdomyosarcoma serum and stained with conjugate. Many pleomorphic cellular elements fluoresce. These are presumably tumor elements with myosin synthesizing capacity. × 140.

FIG. 20. A nephroblastoma consists mostly of dense cells with darkly staining nuclei. Neoplastic pseudo-tubules in upper right-hand corner of photo were present in only a few areas. H&E stain. × 160.

FIG. 21. A nephroblastoma demonstrates numerous pseudo-tubules between dense clumps of other neoplastic cells. H&E stain. × 160.

FIG. 22. The same tissue shown in Figure 21 following irradiation and chemotherapy. There are large (50 to 100 μ) round to oval cells with 1 to 3 nuclei. Histochemical studies (Gomori's trichrome and phosphotungstic hematoxylin acid stains) suggested embryonal muscle cells. H&E stain. × 320.



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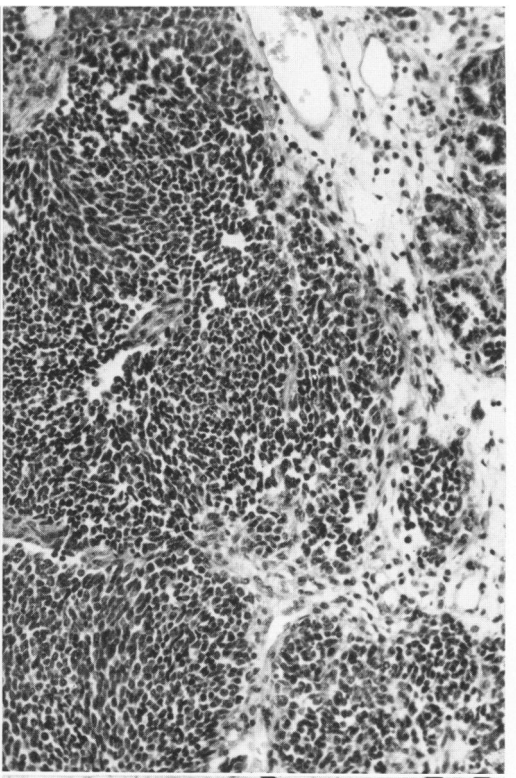

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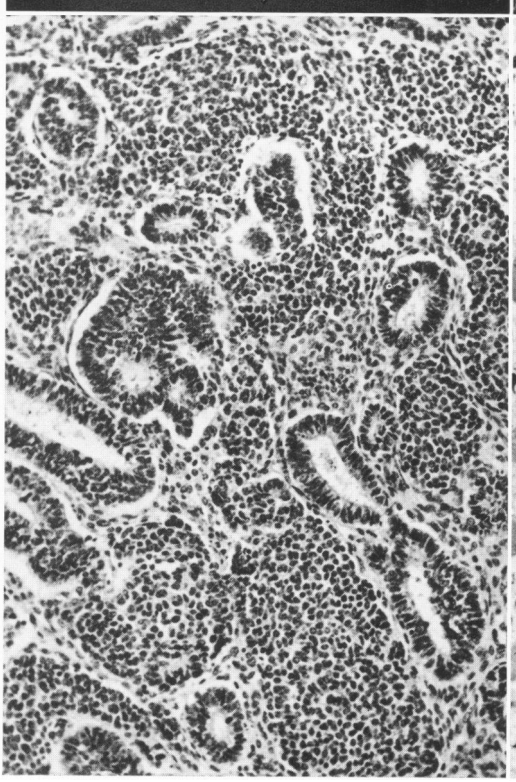

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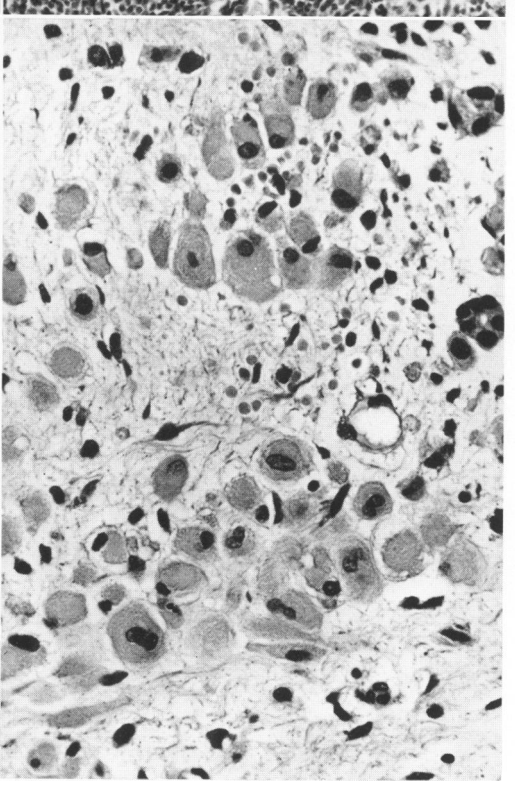

22